The exchangeable calcium pool: physiology and pathophysiology in chronic kidney disease

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Abstract

Excessive soft tissue and vascular calcifications are typical complications of chronic kidney disease (CKD) and disorders of phosphate homeostasis are considered to be a major contributor to the pathogenesis. However, at least in some individuals, calcium administration also increases the risk, and furthermore, it is widely accepted that there is a link between bone disease and vascular pathology.

In this review, we discuss the role of the bone exchangeable calcium pool (ECP) in the acute regulation of the serum calcium concentration ($Ca_s$) in health and CKD. This pool is able to buffer an acute calcium load as well as to maintain a stable $Ca_s$ during acute calcium deprivation. Indeed, the minute-to-minute regulation of $Ca_s$ appears to depend exclusively on this mechanism without any obvious contribution of other factors like parathyroid hormone, which nonetheless define the $Ca_s$ steady state set point. It is tempting to speculate that a reduction of the bone ECP plasticity in some patients with CKD leads to short-lasting increases in $Ca_s$ above the individual mid- to long-term set point as observed during haemodialysis or after the ingestion of calcium-containing phosphate binders. This could contribute to and partially explain the propensity of these subjects to develop extraosseous calcifications. An improved understanding of the processes involved and the availability of new techniques to assess the capacity of this pool, at least in dialysis patients, will make this area an attractive target for new investigations.

Keywords: calcium homeostasis; chronic kidney disease; CKD–MBD; exchangeable calcium pool; extraosseous calcification

Introduction

While disturbances of phosphate homeostasis are considered to be one of the primary factors responsible for vascular calcification in patients with chronic kidney disease (CKD), evidence suggests that administration of excessive calcium (either via administration of calcium-containing phosphate binders or via a dialysate with a high calcium concentration) might be an additional important contributor. Young dialysis patients with coronary artery calcification as determined by electron beam computerized tomography are characterized by a higher level of daily calcium intake when compared to those without [1]. However, some CKD patients do not show calcification despite a high calcium exposure. In the study by Goodman et al., low serum alkaline phosphatase levels were also associated with a greater risk, and subsequent studies pointed out that patients with low-turnover bone disease might be especially susceptible to the harmful effects of calcium loading.
The recent KDIGO guidelines also reconcile this finding in their recommendations concerning phosphate-binder therapy [2]. However, the association between bone turnover and progression of vascular calcification is also far from being absolute, as elegantly shown in a study of Barreto et al. [3]. Even though the risk of progression of coronary artery calcification within a 1-year follow-up period was higher in patients with biopsy-proven low turnover bone disease also some patients with osteitis fibrosa progressed as well as individuals who changed from low- to high-turnover disease. Hence, additional factors should be considered. As far as calcium homeostasis is concerned, an observational study by Block et al. [4] suggested that a low (steady state) serum calcium concentration (Cas) might be protective. Trials using non-calcium-containing phosphate binders have shown a reduced progression of vascular calcification and concluded that the reduction of the total calcium load, and thus a negative or at least less positive calcium balance, might be desirable [5], but even these results have not been reproduced by others [6].

A so far mostly neglected fact is that calcium, when provided during dialysis or by calcium-based phosphate binders during meals in CKD, enters the body rather rapidly. Hence, as the total amount of calcium in the extracellular fluid (ECF) is low and the concentration is tightly regulated just like in healthy individuals, mechanisms must be in place in CKD subjects to either acutely excrete or at least to safely deposit calcium intermittently in order to avoid acute hypercalcaemia (this problem also has to be solved with other ions like potassium, which, after gastrointestinal uptake, is acutely transferred from the ECF to the intracellular space to avoid deleterious bouts of hyperkalaemia). Especially in carnivores, where large meals at infrequent intervals lead to formidable loads of calcium (and phosphate) per body weight, the importance of rapid calcium (and phosphate) deposition or disposal becomes evident.

In this paper, we will review the mechanisms of the acute control of Cas and conclude that a rapidly exchangeable calcium pool (ECP) in the bone has to contribute to a large extent. Furthermore, we will discuss how changes in this pool in CKD might lead to transient episodes of increases of the Ca, above the individual mid- to long-term set point after gastrointestinal uptake or positive dialysate—blood calcium mass transfer, which could be an additional risk factor that predisposes to vascular calcification.

However, this concept does not challenge any of the established risk factors and pathophysiologic mechanisms associated with vascular calcification [7–11].

**Acute versus mid- and long-term regulation of the Cas**

The body of a healthy adult contains ~25 000 mmol (~1 kg) of calcium. More than 99% is part of the mineral component of the bone, <1% can be found in the ECF. The concentration of calcium in the ECF, and thus Ca, is maintained within a very narrow range. As even small deviations impair a variety of cellular functions, the regulation within an individual is even more strict and, at least partially, dependent on genetic predisposition [12]. Over the last decade, our understanding of the regulatory mechanisms has improved greatly, especially after the description of the calcium-sensing receptor (CaSR) by Brown et al. [13] in 1993. The CaSR acts through G-protein-coupled signal transduction pathways that modulate parathyroid hormone (PTH) secretion via a negative feedback depending on Ca,. In hypocalcaemia, for example, a CaSR-mediated stimulatory effect on the rate of PTH secretion leads to calcium transfer to the ECF from the kidneys, bone and the gastrointestinal tract. The response of the parathyroid glands to hypocalcaemia can be subdivided into several time-dependent components. Within seconds, preformed PTH is released from secretory granula, within 15–30 min, intracellular PTH degradation is reduced and more PTH is available for secretion. Hypocalcaemia persisting for >3–12 h leads to an increase in PTH messenger RNA, which is followed by hypertrophy and proliferation of parathyroid cells within days to weeks [14]. The response of the kidneys to increased circulating levels of PTH includes phosphaturia, enhanced transtubular reabsorption of calcium (via synthesis of calbindin 28) and increased formation of active vitamin D. The latter stimulates the transcellular intestinal absorption of calcium (via calbindin 9, the paracellular absorption on the contrary does not seem to be actively regulated) and phosphate and also enhances, together with PTH, the release of calcium and phosphate from the bone. So far, no direct effect of PTH on intestinal calcium or phosphate absorption has been demonstrated.

Although these mechanisms definitely account for mid- to long-term Cas regulation (hours to weeks), their contribution to the minute-to-minute homeostatic mechanisms are far less clear. Bronner and Stein [15] calculated the ECF half-life of an external calcium load to be 14.5 min in rats and similarly, a very rapid and efficient counter-regulatory response to hypocalcaemia has been described. The complex regulatory networks described above, which include vitamin D or calbindin synthesis or degradation, are far too slow to be able to account for these rapid acute responses. Accordingly, the uptake via the gastrointestinal system is able to provide calcium to maintain bone mass [16], however, intestinal participation in the acute regulation of Cas has never been demonstrated [17]. Indeed as shown by Nordin et al. [18], calcium malabsorption alone does not even cause secondary hyperparathyroidism. Lewin et al. [19] showed that the rapid serum calcium recovery phase observed after the induction of acute hypocalcaemia by ethylene glycol tetraacetic acid (EGTA, a calcium chelator) infusion does not depend on the presence or absence of kidneys either. Concerning the renal contribution to the correction of acute hypercalcemia, Kotchen et al. [20] conducted a study where they infused calcium chloride into dogs for 45 min at a rate of 7.5 μequal/kg body weight/min (mean body weight 17.8 kg; thus total amount of calcium infused during the experiment was ~6000 μequal). The infusion moderately increased Ca, but did not change glomerular filtration rate. Renal calcium excretion rose significantly from 1 to 6 μequal/min (or ~270 μequal/45 min). Thus, at least in this experiment, only ~5% of the infused calcium was acutely excreted by the kidney. In the same study, rats were placed on a high calcium diet via drinking water for 17 days. At the end of the
observation period, Ca₄ concentration was identical in control animals on regular calcium intake and chronically calcium-loaded rats [20], however, in the latter, urinary calcium excretion was significantly elevated. The results of this experiment demonstrate that acute and mid- to long-term regulation of calcium homeostasis are different processes with the kidney definitely being the main regulator of the mid- to long-term steady state Ca₄ concentration. Nonetheless, obviously other mechanisms than the kidney account for the rapid correction of Ca₄.

Hence, the skeleton remains as the only compartment to provide the capacity for a rapid response to changes in Ca₄. In the bone, mechanisms responsible for calcium entry and exit are generally perceived to be dependent on cellular events. These include intracellular synthesis of collagen molecules and fibrils in osteoblasts, extrusion of these fibrils and alignment of many such fibres to form extracellular matrix, a proportion of which undergoes mineralization i.e. the deposition of brushite and octacalcium phosphate, which ultimately mature to hydroxyapatite. In the case of bone resorption and calcium release, multinucleated osteoclasts release protons into the extracellular milieu as well as lysosomal enzymes that solubilize bone salt and lyse the matrix. However, treatment with bisphosphonates does not affect the recovery from acute hypocalcaemia in patients with osteoporosis and also cannot prevent the development of phosphate depletion-induced hypercalcaemia [21, 22]. Based on these findings, the contribution of cell-mediated bone events, mainly structural remodeling and (peri)osteocytic osteolysis, in the short-term regulation of Ca₄ is at least questionable [23].

Nonetheless, it is still widely assumed that PTH, which is secreted within seconds after the introduction of hypocalcaemia and has a very short half-life, is also involved in the minute-to-minute regulation of Ca₄, although this concept is massively challenged by several experiments [19, 24]. When normal rats are given a large dose of PTH, no increase in Ca₄ is observed within 60 min. This is in line with the findings of Albright et al. [25], who observed in the late 1920s that the phosphaturic effect of PTH precedes its calcemic effect. In contrast, a transient fall in Ca₄ in the seconds following PTH administration has been previously described in dogs and attributed to an intracellular calcium shift (ionophore effect) [26]. However, later on, rats given PTH develop hypercalcaemia and hypophosphatemia in a strictly dose-related manner. This time course fits nicely to observations showing that PTH administration increases the metabolic activity of osteoclasts after ~3 h. A second phase of PTH action, which includes de novo protein synthesis and an increase in the number of osteoclasts, is noted only after 24 h [27]. In line with the idea that PTH is rather a medium to long-term regulator of Ca₄ and definitely the determinant of the individual Ca₄ set point, is also the fact that rats that underwent parathyreoidectomy (PTX) maintained a normal and stable Ca₄ for 2 h despite no detectable serum PTH. When acute hypocalcaemia was induced in normal animals by infusion of EGTA, 10 min after cessation of EGTA Ca₄ increased significantly again (despite the fact that the EGTA–Ca complexes were absolutely stable and did not dissolve) and reached almost basal levels after 60 min. Most intriguingly, however, when EGTA was given to rats after PTX, Ca₄ recovered in a similar manner as observed in normal animals [24]. This clearly indicates that the rapid release of PTH observed after induction of hypocalcaemia is not necessary for the immediate recovery of Ca₄.

Involvement of the bone in acute Ca₄ regulation independent of cellular remodelling has been suggested repetitively [15, 28–30] but has not received much attention. Ray et al. showed that ~5% of the resting cardiac output flow to the skeleton and almost 50% of radioactive calcium that entered the bone was cleared within a single bone passage. As Ca₄ remained unchanged [15, 31], an extremely high calcium exchange flux between bone and ECF has to take place even under steady state conditions, the amount (~6000 mg/day) exceeding the amount of calcium mobilized by bone remodelling (~400 mg/day) many times [28, 29, 32]. These observations prove the existence of a rapid ECP in the bone, which is able to buffer acute deviations of Ca₄.

Unfortunately, it is hitherto very difficult to assess bone metabolism in healthy individuals and CKD patients as conventional strategies like quantitative computed tomography or dual X-ray absorptiometry are very unreliable [33]. Bone biopsy is still considered the gold standard diagnostic procedure, but nonetheless, even this invasive procedure does not provide information on the ECP. Fortunately, at least in dialysis patients, new approaches have become available.

Recently, the analysis of data reported by Hou et al. [34] led to the conclusion that this ECP is also required to avoid fluctuations in Ca₄ in the event of positive or negative Ca mass balance during dialysis [32]. A kinetic model showed either sequestration or mobilization in/from this pool and furthermore for the first time allowed a quantitative assessment of the acute buffering capacity of the ECP based on expected versus observed changes in Ca₄ in response to a positive or negative calcium mass transfer during a dialysis session [32]. These calculations might also allow testing of the plasticity of the ECP when exposing patients sequentially to increasing or decreasing dialysate calcium concentrations. However, this method does not allow the discrimination of whether the ECP accessed in dialysis patients is located at the level of the bone or at some other (extraskeletal) compartment.

**What is the location and nature of the ECP in the bone?**

Despite the data clearly indicating that a rapid ECP exists in the bone compartment, its exact nature remains a matter of discussion. The bone contains a variety of calcium salts (e.g. brushite, octacalcium phosphate, amorphous calcium phosphate, whitlockite, hydroxyapatite). While hydroxyapatite [HA, Ca₁₀ (PO₄)₆(OH)₂] has the lowest solubility/highest calcium-binding affinity, brushite’s (CaHPO₄ 2H₂O, dicalcium phosphate dihydrate) solubility is the highest of all calcium salts, almost equivalent to the total/ionic Ca₄ [30]. Although the ECF is in direct contact with bone surface [35], the equilibrium between...
HA, the major inorganic calcium-containing constituent of bone matrix, and ECF is poorly understood [36]. Compared to the rather insoluble HA, the ECF is supersaturated with calcium. This would result in a massive and persistent gradient of calcium towards the bone that would favour indefinite HA growth [28, 30] but would totally deplete the ECF from calcium. Thus, a barrier against free diffusion is mandatory [17]. For some time, it was proposed that this barrier is maintained by lining cells on the surface of the bone (so-called bone membrane), which operate to pump calcium actively from HA to the ECF. However, these theoretical considerations have never been confirmed experimentally [37] but rather brushite has been proposed to play a central role [17, 38]. When artificially produced HA is brought into contact with a sodium chloride-containing fluid, brushite spontaneously forms on the surface. Interestingly, in vitro, this modification also enhances the binding capacity of the surface for preosteoblasts [36]. In vivo, brushite may not only drastically reduce the calcium gradient from the ECF towards HA but also, due to its high solubility, might act as ECP. Both rapid correction of $C_{a}$ deviations as well as the high calcium bone—ECF exchange fluxes in steady state may be due to purely physicochemical reactions (dissolution/precipitation) involving brushite.

Interestingly, brushite is mainly found in the vicinity of newly formed bone [39] but becomes more and more hydrolysed to an insoluble phase over time as the HA content increases [40]. This observation has been corroborated by the fact that brushite cement, despite its good biocompatibility, tends to convert into apatite over time [40, 41]. This conversion can be slowed by the presence of so-called noncollagenous bone proteins (NCBPs), which bind directly to HA at the interface between bone mineral and ECF [30]. While some of them, such as osteopontin, are localized ahead of the mineralization front and are necessary for initiation of mineralization, others, like osteocalcin and osteonectin, are present in the fully mineralized matrix [42]. Thus, NCBPs are involved both in primary and secondary mineralization. More than 30 years ago, osteocalcin and osteonectin were extracted from cortical bones and added to a buffered solution surrounding HA, which led to an increase in the solution’s calcium concentration [40, 43, 44]. Thus, it was proposed that these NCBPs increase bone mineral solubility in vitro by preventing apatite formation (crystal poison) [40], which in turn might be due to brushite stabilization [29, 40]. Contribution of these two calcium-binding proteins to the regulation of size and speed of HA crystal formation has been suggested [42].

Osteonectin (SPARC), a phosphorylated, alkaline phosphatase-sensitive glycoprotein, has been linked to the regulation of bone mineralization [45, 46] but is also implicated in extrasosseous calcification and therefore is not restricted to bone tissue [45]. Osteocalcin, the most abundant NCBP [47], contains three vitamin K-dependent γ-carboxylated glutamic acid (Gla) residues [48] and is synthesized by osteoblasts only [49]. Its expression is regulated at the transcriptional level by hormones like active vitamin D [50] and retinoic acid [51], growth factors like tumour necrosis factor-alpha [52], basic fibroblast growth factor [53] or glucocorticoids [54]. Osteocalcin is considered a negative regulator of bone formation as its deficiency increases bone formation [49], density and thickness [55]. Osteocalcin, also referred to as bone Gla protein, is closely related to another GLA family protein, namely matrix Gla protein, that has already been shown to be an inhibitor of calcification [56]. Interestingly, the fact that synthesis of Gla proteins is vitamin K dependent might explain the association between oral anticoagulant therapy and bone disease as well as catastrophic soft tissue calcifications in the form of calciphylaxis in patients with CKD [57, 58].

Besides NCBPs pyrophosphate, a previously described inhibitor of mineralization [59] and vascular calcification [60], is another regulator of the interaction between HA and the soluble calcium pool in the bone [61]. Accordingly, we were able to show that a polymorphism in the gene encoding the ectonucleotide pyrophosphatase/phosphodiesterase-1, which generates inorganic pyrophosphate, is associated with coronary artery calcification and increased aortic stiffness [62].

However, as pointed out by Talmage et al. [30], the equilibrium $C_{a}$ that would be reached if an isolated spontaneous reaction would occur between HA, the brushite/NCP component of the bone and the ECF, is predicted to be ~3.5 mg/dL, still not high enough to maintain normal activity of many physiological processes. Therefore, it was postulated that PTH is able to increase the solubility of the layer to a value that is normally seen in vivo. But again, PTH in this regard is more a determinant of the mid- to long-term regulation of $C_{a}$ rather than a hormone that affects acute changes in the concentration of calcium in the ECF.

How could CKD affect the ECP?

When Kaye et al. infused phosphate into anuric dialysis patients and achieved hyperphosphataemia, they observed a reduction in $C_{a}$, Elegant labelling studies demonstrated that this was not due to precipitation of calcium and phosphate in the ECF but rather was caused by a reduction of calcium efflux from the ECP [38]. Similar results were obtained by Raisz et al. [63]. This observation is also an attractive explanation for the decrease in $C_{a}$ in CKD with hyperphosphataemia independent from changes in active vitamin D status. A pathophysiological explanation for the reduced calcium efflux during hyperphosphatemia is provided by the work of Roy et al. [64], who demonstrated that NCBP binding to HA was increased by calcium but decreased by phosphate in a dose-dependent manner. While calcium effects on the NCBP—HA binding are most likely due to conformational changes, the exact mechanism by which phosphate affects this interaction remains unclear [64]. Interestingly, reduced bone mineral dissolution [17, 65] and reduced brushite solubility [29, 38] have been proposed by various other authors to result from an increase in phosphate levels. In line with these findings are data from animal experiments showing that hypercalcaemia, which can be induced by phosphate depletion, is a
consequence of an increased mobilization of calcium from the bone exchangeable pool [66]. As the latter results were not altered by bisphosphonate therapy [21], it was also concluded that physicochemical exchange rather than specific effects on osteoclasts is responsible [21]. In summary, there is a clear link between phosphate homeostasis and the size and/or accessibility of the ECP, which might be of importance in CKD.

Another typical complication of CKD, which might affect the ECP, is acidosis (Figure 1) as a decrease in pH increases brushite solubility [67]. Bushinsky et al. measured the calcium efflux from neonatal mouse calvaria at a pH of 7.4 and 7.1 in the presence and absence of calcitonin, an inhibitor of osteoclast activity. At physiological pH, blocking of cellular activity stopped the net transfer of calcium to the medium within the first 3 h; under conditions of acidosis, however, calcium efflux continued although at a smaller magnitude [68]. While acidosis (e.g. during starving periods) leads to calcium efflux from bone, it is tempting to speculate that alkalosis, especially postprandial (alkaline tide), might help to reduce bone calcium efflux or even increase calcium deposition in the event of large meals.

At least in haemodialysis patients, active vitamin D might also regulate the size and/or accessibility of the ECP as suggested by Pahl et al. During vitamin D substitution, Ca₉ decreased and increased more rapidly and to a greater extent during low and high dialysate calcium dialysis, respectively, when compared to a period without therapy [69]. This effect might result from a reversible direct effect of active vitamin D on the ECP in bone [50] (Figure 1).

Whether PTH per se directly affects the size and/or accessibility of the ECP is still a matter of discussion. As elegantly shown by Parfitt et al. [29], subjects with primary hyperparathyroidism (pHPT) do respond to a hypocalcaemic stimulus, as far as Ca₉ is concerned, identically as normal controls. However, despite altered Ca₉ and physicochemical calcium gradients between ECF and ECP, steady state Ca₉ remains stable in patients with stable pHPT or hypoparathyroidism. Thus, although not directly involved in Ca₉ regulation in the case of acute deviations in general, it seems that the level of serum PTH in these diseases also determines the steady state levels of Ca₉. Nonetheless, an indirect effect of PTH on the ECP seems likely as bone remodelling affects the HA/brushite ratio very well and as brushite converts into apatite over time, bone aging per se decreases the ECP (Figure 1). Under normal conditions, this is balanced by the fact that newly formed bone is rich in brushite [29]. Pinto et al. demonstrated in canines that low affinity (exchangeable) calcium-binding sites, located mostly at the bone surface, decrease from puberty to adolescence. A further age-related reduction in the ECP might result from reduced bone vascularization (Figure 1) [70].

Accepting brushite’s role in Ca buffering, low-bone turnover (e.g. adynamic bone disease) impairs buffering of calcium loads [71, 72] and this would support the theory that certain patients with CKD are much more prone to develop vascular calcification after calcium loading than others (Figure 1). Also, reports that calcium loading in patients with osteoporosis has harmful effects could be explained by this mechanism [73, 74]. In line with these data are also observations by Karohl et al. [75], who showed that the calcium mass transfer during dialysis is dependent not only on the dialysate calcium concentration used but also the bone remodelling state of the patient.

In summary, in health, the bone ECP is used almost exclusively for rapid correction of acute Ca₉ deviations in order to maintain the steady state Ca₉ set point that is determined by factors such as PTH and active vitamin D. At the same time, these and other factors (listed in Figure 1) affect size and/or plasticity of the bone ECP, and
thus, indirectly contribute to acute Ca\textsubscript{r} regulation. This dependency could partially explain the propensity of certain patients with CKD or specific bone diseases to develop extraosseous calcification (i.e. the generation of an extraosseous ECP).

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